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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
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Additional inventors are being named on the 1 separately *numbered sheets* attached hereto

TITLE OF THE INVENTION (280 *characters max*)

MCEMP1, A HUMAN MAST CELL-EXPRESSED MEMBRANE PROTEIN

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

[X] Specification *Number of Pages*	29	[ ] CD(s), Number	
[X] Drawing(s) *Number of* sheets	6	[ ] Other (specify)	
[ ] Application Data Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[X]	Applicant claims small entity status. See 37 CFR 1.27.	FILING FEE AMOUNT ($)
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No

[ ] Yes, the name of the U S Government agency and the Government contract number are. _____

Respectfully submitted

SIGNATURE Cheryl A. Lilestrand

Date 01/03/2002

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REGISTRATION NO. *(if appropriate)* 45,275

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Number 1 of 1

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U.S. PROVISIONAL APPLICATION

FOR:

MCEMP1, a Human Mast Cell-Expressed Membrane Protein

BY:

Kang Li, Yucheng Li, Shen-Wu Wang, Guanghui Hu,

and Zhengbin Yao

BACKGROUND OF THE INVENTION

Mast cells originate from hematopoietic stem cells in the bone marrow but complete their development only after they migrate into diverse peripheral tissues. Mature mast cells express the high-affinity IgE receptor FcεRI on their surface, which can be activated by IgE cross-linked with specific allergens. Mast cells can also be activated by IgE independent mechanisms. C3a, and C5a have been shown to activate mast cells *in vivo* and calcium ionophores, such as A23187, have been shown to activate mast cells *in vitro*.

Mast cells contain a wide variety of preformed secretory inflammatory mediators such as histamine, tryptase, peroxidase, and neutrophil chemotactic factor. Upon activation, mast cells release these mediators, as well as newly synthesized lipid mediators, and secrete both induced immunomodulatory and proinflammatory cytokines including TNFα, IL-4, IL-13, IL-5, IL-10, and chemokines.

It is well known that human mast cells play critical roles in the pathogenesis of many allergic diseases, such as asthma, atopic dermatitis, and other allergic diseases. The mediators released by mast cells are responsible for most of the early events in allergic reactions, and through cytokine production and other mechanisms, contribute to the expression of late-phase reactions and chronic allergic inflammation. Mast cells have also been observed in a multitude of neoplastic, fibrotic, and inflammatory processes, such as lymphoproliferative disorders, interstitial lung disease, and the synovium in rheumatoid arthritis. Furthermore, the number of mast cells is highly elevated in other inflammatory diseases, such as inflammatory bowel disease. Recent studies suggest that mast cells play a role in the progression of heart failure. Mast cells

are found in human heart and numbers are increased in the failing heart and their density is higher in ischemic cardiomyopathy.

Since mast cells play such a critical role in allergic reactions and other inflammatory diseases, therapeutic agents that can regulate mast cell differentiation, proliferation, adhesion, maturation, and activation should provide beneficial effects to many of these conditions.

SUMMARY OF THE INVENTION

The invention relates to a cDNA sequence (SEQ ID NO:1), and functional variants thereof, that encode mast cell-expressed membrane protein 1 (MCEMP1) (SEQ ID NO:2), which is highly expressed in human mast cells and lung.

The present invention includes full-length MCEMP1 polypeptides, as well as biologically active fragments and variants thereof. Soluble polypeptides comprising the extracellular domain of MCEMP1 or a fragment thereof are among the biologically active fragments provided. Likewise, soluble polypeptides derived from the extracellular domain of human MCEMP1 that are capable of binding the MCEMP1 ligand are encompassed by the present invention. Such soluble polypeptides, fragments, and variants are described in more detail below.

The invention also relates to antagonist and agonist molecules, including monoclonal antibodies, peptides, proteins, nucleic acids, or small bioorganic molecules, which specifically bind to MCEMP1, inhibiting or activating the expression or action of MCEMP1. These antagonistic and agonistic molecules can also block the binding of the natural ligand for MCEMP1 and include the soluble form of MCEMP1, as well as monoclonal antibodies that bind MCEMP1, and can be used for the treatment of mast

cell-mediated immunological diseases, such as asthma, allergic disease, chronic obstructive pulmonary disease (COPD), Crohn's disease, and rheumatoid arthritis.

The invention includes recombinant forms of MCEMP1, which can be expressed in a variety of cell types and can be used in assays for the screening for monoclonal antibodies and small molecule inhibitors. The invention also includes cells and hybridomas that express such molecules. In addition, the recombinant protein, monoclonal antibodies that bind MCEMP1 or other molecules of the invention that bind to MCEMP1, can be used in the diagnostic detection of MCEMP1.

In addition, the invention relates to expression vectors that contain the nucleic acid sequence encoding MCEMP1 or a biologically active variant thereof.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 depicts the nucleotide sequence (SEQ ID NO:1) and the deduced amino acid sequence (SEQ ID NO:2) of MCEMP1.

Fig. 2 depicts genomic organization and alternative splicing of MCEMP1.

Fig. 3A depicts relative expression (real fold difference) of MCEMP1 in tissues as analyzed by quantitative RT-PCR analysis.

Fig. 3B depicts relative expression (real fold difference) of MCEMP1 in various primary cells and cell lines.

Fig. 4 depicts the expression of recombinant protein of MCEMP1 as analyzed by Western blot analysis and the association of MCEMP1 with cellular membranes.

Fig. 5 depicts the subcellular expression of MCEMP1 in transfected 293T cells as analyzed by flow cytometry.

DEFINITIONS

"Nucleic acid sequence", as used herein, refers to an oligonucleotide, nucleotide, or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin, which may be single- or double-stranded, and represent the sense or antisense strand.

Similarly, "amino acid sequence" as used herein refers to an oligopeptide, peptide, polypeptide, or protein sequence, and fragments or portions thereof, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

"MCEMP1", as used herein, refers to the amino acid sequences of substantially purified MCEMP1 obtained from any species, particularly mammalian, including bovine, ovine, porcine, murine, equine, and preferably human, from any source whether natural, synthetic, semi-synthetic, or recombinant.

A "variant" of MCEMP1, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, e.g., replacement of glycine with tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without

abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

A "deletion", as used herein, refers to a change in either amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent.

An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively, as compared to the naturally occurring molecule.

A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

The term "biologically active", as used herein, refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic MCEMP1, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "antagonist" is used herein in the broadest sense to include any molecule that blocks, prevents, inhibits, or neutralizes the activity of MCEMP1. In a similar manner, the term "agonist" is used herein to include any molecule that promotes, enhances, or stimulates the activity of MCEMP1. Non-limiting examples include proteins, peptides, glycoproteins, glycopeptides, glycolipids, polysaccharides, oligosaccharides, nucleic acids, bioorganic molecules, peptidomimetics, antibodies, pharmacological agents and their metabolites, transcriptional and translation control sequences, and the like.

The term "modulate", as used herein, refers to a change or an alteration in the biological activity of MCEMP1. Modulation may be an increase or a decrease in protein activity, a change in binding characteristics, or any other change in the biological, functional, or immunological properties of MCEMP1.

The term "mimetic", as used herein, refers to a molecule, the structure of which is developed from knowledge of the structure of MCEMP1 or portions thereof and, as such, is able to effect some or all of the actions of novel human MCEMP1 receptor-like molecules.

The term "derivative", as used herein, refers to the chemical modification of a nucleic acid encoding MCEMP1 or the encoded MCEMP1. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group. A nucleic acid derivative would encode a polypeptide that retains essential biological characteristics of the natural molecule.

The term "substantially purified", as used herein, refers to nucleic or amino acid sequences that are removed from their natural environment, isolated or separated, and are at least 60% free, 75% free, or 90% free from other components with which they are naturally associated.

"Amplification" as used herein refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies well known in the art (Dieffenbach, C. W. and G. S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y.).

The term "homology", as used herein, refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). A partially

complementary sequence is one that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid; it is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous sequence or probe to the target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% identity); in the absence of non-specific binding, the probe will not hybridize to the second non-complementary target sequence.

As known in the art, numerous equivalent conditions may be employed to comprise either low or high stringency conditions. Factors, such as the length and nature (DNA, RNA, base composition) of the sequence, nature of the target (DNA, RNA, base composition, presence in solution or immobilization, etc.), and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate and/or polyethylene glycol) are considered. The hybridization solution may be varied to generate conditions of either low or high stringency different from, but equivalent to, the above listed conditions.

The term "stringent conditions", as used herein, is the "stringency" which occurs within a range from about Tm-5°C. (5°C below the melting temperature (Tm) of the probe) to about 20°C to 25°C below Tm. As will be understood by those of skill in the art, the stringency of hybridization may be altered in order to identify or detect identical or related polynucleotide sequences.

The terms "specific binding", as used herein, in reference to the interaction of an antibody and a protein or peptide, means that the interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) on the protein. In other words, the antibody is recognizing and binding to a specific protein structure rather than to proteins in general. For example, if an antibody is specific for epitope "A", the presence of a protein containing epitope A (or free, unlabeled A) in a reaction containing labeled "A" and the antibody will reduce the amount of labeled A bound to the antibody.

As used herein, the term "antibody" refers to intact molecules as well as fragments thereof, such as Fab, F(ab'), $F(ab')_2$, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind MCEMP1 polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or peptide used to immunize an animal can be derived from the transition of RNA or synthesized chemically, and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin and thyroglobulin. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

DETAILED DESCRIPTION OF THE INVENTION

The gene encoding MCEMP1 is differentially expressed in mast cells derived from $CD34^+$ progenitor cells and was cloned using the subtractive hybridization and screening method described below. MCEMP1 is predicated to be a transmembrane protein of 187 amino acids based on sequence analysis of the nucleic acid sequence SEQ ID NO:1. The putative protein sequence contains a transmembrane domain at amino acid residue 83 to amino acid residue 105 (aa83-aa105) of SEQ ID NO:2.

Quantitative real-time RT-PCR profiling showed that the MCEMP1 mRNA was highly expressed in primary mast cells (See Fig. 3B). MCEMP1 was also highly expressed in human lung, as compared with brain, heart, kidney, liver, and tracheal tissues (See Fig. 3A). When expressed in 293T cells, recombinant MCEMP1 was expressed as an approximately 29 to 35 kDa protein associated with the membrane fraction and localized on the cellular membrane with an N-terminal region extending outside of the membrane (See Fig. 4 and 5).

Identification of MCEMP1

MCEMP1 was identified by subtractive hybridization using human mast cell mRNA as a tester and a combination of mRNAs from human THP-1 (~45%), Daudi (~35%) and TF-1 (~20%) cell lines as drivers. Approximately 45 subtracted clones were isolated, sequenced, and used to search for matches in the publicly available nucleotide/protein databases. A cDNA clone comprising a 369 base pair (bp) insert isolated by the subtractive hybridization only matched to a number of EST clones that contain partial cDNA sequences, but it showed no significant homology to any cDNA sequences that encode known or predicted proteins in the GenBank database.

Two oligonucleotide primers

5' CTCCCAGAAAGGTGATGAA SEQ ID NO:3, and

5' TCTGTCTTTTGTGCGGCGTCAT SEQ ID NO:4,

based on the 369 bp insert sequence were synthesized and used to screen a human peripheral blood leukocyte cDNA library (OriGene Technologies, Inc., Rockville, MD.). Several cDNA clones were isolated and sequenced. Three alternative splicing forms of mRNAs were identified by comparing the cDNA sequences with genomic sequences in the GenBank database (Fig. 2). Two of cDNA clones (7B and 2G, see Fig. 2) represent aberrant mRNA transcripts, because the putative translation product in all three reading frames would be aborted by stop codons. However, the majority of the cDNA clones predict a protein product, which is derived from seven exons in the MCEMP1 gene (Fig.1 and Fig.2). One such cDNA clone (9E) contains a full length coding region comprised ofabout450 bp 5' untranslated region and about 726 bp 3' untranslated region.

In addition, a cDNA clone was also obtained from an HMC-1 cell line by RT-PCR using an oligo primer covering the starting methionine codon

5'GACCATGGAAGTGGAGGAAATCTAC SEQ ID NO:5 and

an oligo primer covering the stop codon,

5' GCAGGTGCAGCCCCATCTT SEQ ID NO:6.

These cDNAs are predicted to encode a polypeptide of 187 amino acids (Fig. 1). The predicted starting methionine codon is associated with a perfect Kozak sequence motif (ACCATGG), making it optimal for translation initiation. An allelic variation was found

at amino acid residue167 (Ile ←→ Val) among the cDNA clones, which is caused by a single nucleotide change at the first codon position (ATT ←→ GTT).

Computer-assisted analysis predicts that MCEMP1 has a transmembrane sequence located at amino acid residues 83 to105. There does not appear to be a discernable N-terminal hydrophobic leader sequence. The predicted molecular mass for MCEMP1 is 21 kDa. A comparison of both nucleotide and amino acid sequences with GenBank or European Molecular Biology Laboratory databases revealed that it shares 37% amino acid identity with BAB25183, a putative mouse sequence identified by The RIKEN Genome Exploration Research Group Phase II Team and the FANTOM Consortium. A 3-dimentional structure prediction was carried out using a threading-based fold recognition method (Kelley et al., J. Mol. Biol. 299:499-520 (2000)). Briefly, using a library of known protein structures, the MCEMP1 sequence was "threaded" and scored for compatibility. Four components were used in the scoring system: 1D and 3D sequence profiles coupled with secondary structure and salvation potential information. Since the prediction of transmembrane helix showed that MCEMP1 contained a transmembrane segment (aa83-aa105), fold recognition process has been applied on the N-terminal part (aa1-aa82) and C-terminal part (aa106-aa187) separately in order to improve the accuracy. The results showed that the N-terminal region (aa6-aa65) might adopt an Ig-like β sandwich fold, sharing 21% identity with Ig domain of mouse T-cell receptor α-chain.

Soluble Form of MCEMP1

Soluble forms of the MCEMP1 proteins are provided herein. Soluble MCEMP1 polypeptides comprise all or part of the extracellular domain, but lack the

transmembrane region that would cause retention of the polypeptide on a cell membrane. The soluble MCEMP1 polypeptides that may be employed retain the ability to bind an MCEMP1 ligand. The soluble proteins may include part of the transmembrane region or part of the cytoplasmic domain, if the protein is not retained on the cell surface. Since the MCEMP1 protein lacks a discernable leader peptide, a heterologous signal peptide may be advantageously fused to the N-terminus of soluble MCEMP1 polypeptides to promote secretion thereof. The signal peptide is cleaved from the protein upon secretion from the host cell. The need to lyse the cells and recover the recombinant soluble protein from the cytoplasm thus is avoided.

Soluble proteins of the present invention may be identified (and distinguished from their non-soluble membrane-bound counterparts) by separating intact cells expressing the desired protein from the culture medium, e.g., by centrifugation, and assaying the medium (supernatant) for the presence of the desired protein. The culture medium may be assayed using similar or identical procedures to those described in the examples below.

Soluble forms of the MCEMP1 proteins are advantageous for certain applications, e.g., when the protein is to be administered intravenously for certain therapeutic purposes. In addition, purification of the proteins from recombinant host cells is facilitated, since the soluble proteins are secreted from the cells.

In one embodiment of the invention, a soluble fusion protein comprises a first polypeptide derived from the extracellular domain of MCEMP1 fused to a second polypeptide added for purposes such as facilitating purification or effecting dimer formation. Suitable second polypeptides do not inhibit secretion of the soluble fusion

protein. Examples of soluble polypeptides include those comprising the entire extracellular domain. Representative examples of the soluble proteins of the present invention include, but are not limited to, a polypeptide comprising amino acids of SEQ ID NO:2, wherein the polypeptide is selected from amino acids 1-82 of SEQ ID NO:2, amino acids 6-65 of SEQ ID NO:2, or any fragment thereof that retains the ability to bind MCEMP1 ligand. Truncated forms of the inventive proteins, including soluble polypeptides, may be prepared by any of a number of conventional techniques.

Recombinant MCEMP1

In the case of recombinant proteins, a DNA fragment encoding a desired fragment may be subcloned into an expression vector. Alternatively, a desired DNA sequence may be chemically synthesized using known techniques. DNA fragments also may be produced by polymerase chain reaction ("PCR") to isolate a DNA sequence encoding a desired protein fragment by using oligonucleotide primers comprising sequences that define the termini of the desired fragment. In another approach, enzymatic treatment (e.g., using Bal 31 exonuclease) may be employed to delete terminal nucleotides from a DNA fragment to obtain a fragment having a particular desired terminus.

Isolated, naturally occurring soluble forms of MCEMP1 are also encompassed by the present invention. Such soluble polypeptides may result from alternative splicing of mRNA during expression, or release of a soluble polypeptide from a membrane-bound form of the protein by proteolysis. Oligomeric (multimeric) forms of the inventive proteins are encompassed by the present invention. The terms "inventive

proteins" and "inventive polypeptides" as used herein refer collectively to MCEMP1 proteins or polypeptides of the present invention, as defined by the appended claims.

Fusion proteins are also included in the invention. A gene fusion encoding the fusion protein is inserted into an appropriate expression vector and cells transformed with the expression vector are cultured to produce and secrete the fusion protein.

Variants and Derivatives

As used herein, the term "MCEMP1" includes variants and derivatives that retain a desired biological activity of the native mammalian polypeptide. The variant sequences differ from a native nucleotide or amino acid sequence by one or a plurality of substitutions, deletions, or additions, but retain a desired biological activity such as the ability to bind MCEMP1 ligand (for variants of MCEMP1).

In one embodiment of the present invention, a variant sequence is substantially identical to a native sequence. The term "substantially identical" as used herein means that the amino acid or nucleotide sequence in question is at least 80% identical, or 90 to100% identical, to a reference (native) sequence. The degree of homology (percent identity) may be determined, for example, by comparing sequence information using the GAP computer program described by Devereux et al. (Nucl. Acids Res. 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (J. Mol. Biol. 48:443, 1970), as revised by Smith and Waterman (Adv. Appl. Math 2:482, 1981). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and

Burgess, Nucl. Acids Res. 14:6745, 1986, as described by Schwartz and Dayhoff, eds., Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Alterations of the native amino acid sequence may be accomplished by any of a number of known techniques, e.g., by mutation of the native nucleotide sequences disclosed herein. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence. Following PCR, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Isolated DNA sequences that hybridize to the MCEMP1-encoding nucleotide sequence of SEQ ID NO:1 under moderately stringent or severely stringent conditions are encompassed by the present invention. Moderate stringency conditions refer to conditions described in, for example, Sambrook et al. Molecular Cloning: A Laboratory Manual, 2 ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press, (1989). Conditions of moderate stringency, as defined by Sambrook et al., include prewashing in 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization at about 55°C. in 5X SSC overnight. Conditions of severe stringency include higher temperatures of hybridization and washing. The skilled artisan recognizes that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as the length of the probe. One embodiment of the invention is directed to DNA sequences that will hybridize under severely stringent conditions to a DNA sequence comprising the coding region of a MCEMP1 clone disclosed herein. The severely stringent conditions include hybridization at 68°C. followed by washing in 0.1X

SSC/0.1% SDS at 63°C to 68°C. Among the hybridizing sequences encompassed by the present invention are those encoding a biologically active mammalian MCEMP1 polypeptide. Biologically active polypeptides encoded by DNA sequences that hybridize to the MCEMP1-encoding nucleotide sequence of SEQ ID NO:1 under moderately stringent or severely stringent conditions are encompassed by the present invention.

In one embodiment of the present invention, a variant amino acid sequence comprises conservative amino acid substitution(s) but is otherwise identical to a native amino acid sequence. Conservative substitutions refer to replacement of a given amino acid residue with a residue having similar physiochemical characteristics. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.

The present invention further includes the inventive polypeptides with or without associated native-pattern glycosylation. The recombinant proteins when expressed in yeast or mammalian expression systems (e.g., COS-7 cells) may be similar or significantly different in molecular weight and glycosylation pattern from the corresponding native proteins. Expression of mammalian MCEMP1 polypeptides in bacterial expression systems, such as E. coli, provides non-glycosylated molecules. Variant proteins comprising inactivated N-glycosylation sites are within the scope of the

present invention. Such variants are expressed in a more homogeneous, reduced carbohydrate form.

Naturally occurring variants such as those resulting from alternative mRNA splicing events or proteolytic cleavage are also within the scope of the present invention. Variations attributable to proteolysis include, for example, differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids (which may occur intracellularly or during purification). In one embodiment of the present invention, the inventive proteins may lack certain N- or C-terminal amino acids of the sequences disclosed herein. In certain host cells, post-translational processing will remove the methionine residue encoded by an initiation codon, whereas the methionine residue will remain at the N-terminus of proteins produced in other host cells. Additional variants may be prepared by deleting terminal or internal sequences not needed for biological activity. For example, Cys residues can be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation.

Derivatives of the inventive proteins may comprise moieties such as the chemical moieties attached to the inventive protein. The inventive proteins may be modified by forming covalent or aggregative conjugates with chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups, and the like. Covalent derivatives are prepared by reaction of functional groups of the chemical moiety with functional groups on amino acid side chains or at the N-terminus or C-terminus of the inventive protein. Also provided herein are the inventive proteins comprising detectable labels, diagnostic or cytotoxic reagents attached thereto, including but not limited to

radionuclides, colorimetric reagents, and the like. Other derivatives within the scope of this invention include covalent or aggregative conjugates of the inventive proteins or fragments thereof with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. The inventive proteins can comprise polypeptides added to facilitate purification and identification (e.g., the antigenic identification peptides described in U.S. Pat. No. 5,011,912 and Hopp et al., Bio/Technology 6:1204, 1988; or a poly-His peptide) to enable rapid assay and facilitate purification of the expressed recombinant polypeptide fused thereto.

<u>Making Monoclonal Antibodies</u>

Monoclonal antibodies may be made against MCEMP1 using the hybridoma method first described by Kohler et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal is immunized as described above to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusion agent, such as polyethylene glycol, to form a hybridoma cell (Goding, <u>Monoclonal Antibodies: Principles and Practice</u>, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or

HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP2/0 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for use in the production of human monoclonal antibodies (Kozbor, *J. Immunol.* 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). The mouse myeloma cell line NS0 may also be used (European Collection of Cell Cultures, Salisbury, Wiltshire UK).

Culture medium in which hybridoma cells are grown is assayed for production of monoclonal antibodies directed against the antigen. The binding specificity of monoclonal antibodies produced by hybridoma cells may be determined by immunoprecipitation or by an *In vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies:

Principles and Practice, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (Innis M. et al., In: PCR Protocols. A Guide to Methods and Applications, Academic, San Diego, CA (1990), Sanger, F.S, et al. *Proc. Nat. Acad. Sci.* 74:5463-5467 (1977)). The hybridoma cells serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant production of antibodies is described in more detail below.

In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty, et al., *Nature* 348:552-554 (1990). Clackson, et al., *Nature* 352:624-628 (1991) and Marks, et al., *J. Mol. Biol.* 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks, et al., *Bio/Technology* 10:779-783 (1992)), as well as combinatorial infection and *in vivo*

recombination as a strategy for constructing very large phage libraries (Waterhouse, et al., *Nuc. Acids. Res.* 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; Morrison, et al., *Proc. Nat. Acad. Sci.* USA 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically, such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

Another alternative is to use electrical fusion rather than chemical fusion to form hybridomas. This technique is well established. Instead of fusion, one can also transform a B-cell to make it immortal using, for example, an Epstein Barr Virus, or a transforming gene. (See, e.g., "Continuously Proliferating Human Cell Lines Synthesizing Antibody of Predetermined Specificity," Zurawaki, V. R. et al, in Monoclonal Antibodies, ed. by Kennett R. H. et al, Plenum Press, N.Y. 1980, pp 19-33.)

Humanized and Human Antibodies

A humanized antibody can be chimeric, meaning that only the Fc regions are of human origin, or CDR-grafted, in which the Fc and substantial portions of the

framework regions are of human origin. Humanization can be performed following the method of Winter and co-workers (Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-327 (1988); Verhoeyen, et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, in such "humanized" antibodies, a substantially less than intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. *See, e.g.,* U.S. Patent Nos. 4,816,397; 5,585,089; 5,693,762. Alternatively, transgenic animals (e.g., mice) are available which, upon immunization, can produce a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. Such transgenic mice are available from Abgenix, Inc., Fremont, California, and Medarex, Inc., Annandale, New Jersey. It has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. *See, e.g.,* Jakobovits et al., *Proc. Natl. Acad. Sci.* USA 90:2551 (1993); Jakobovits et al., *Nature* 362:255-258 (1993); Bruggermann et al., *Year in Immunol.* 7:33 (1993); and Duchosal et al. *Nature* 355:258 (1992). Human antibodies can also be derived from phage-display libraries (Hoogenboom et al., *J. Mol. Biol.* 227:381 (1991); Marks et al., *J. Mol. Biol.* 222:581-597 (1991); Vaughan, et al., *Nature Biotech* 14:309 (1996)).

Making Other Compounds of the Invention

Rather than using monoclonal antibodies in diagnostic kits, it may be possible to detect the presence of MCEMP1 by using other compounds that bind to it, wherein the compounds are labeled and able to be detected. Such compounds may be isolated by screening compound libraries and/or peptide libraries. Library members, which are capable of interacting with MCEMP1, can be labeled with a fluorescent marker, or a radioactive marker, using a linker such as a peptide or other covalent chemical conjugate to join the compound with the marker. The resulting labeled compound can be used in a diagnostic kit to indicate the presence of MCEMP1 positive cells, using well-known methods.

Example 1

Quantitative real-time PCR analysis of MCEMP1 mRNA expression:

Two sets of oligonucleotide primers

5'AAGGTGATGAATGAATAGGACTGA 3' SEQ ID NO:7 and

5' CCACCGTGACATGCCGAGACT 3' SEQ ID NO:8

were selected from the MCEMP1 nucleotide sequences using Primer Express 2.0 (Applied Biosystems, Inc.) and were synthesized and used in RT-PCR reactions to monitor the expression of MCEMP1.

Real-time quantitative PCR was performed with the ABI Prism 7900 (Applied Biosystems, Inc.) sequence detection system, using CYBR Green reagents, according to the manufacture's instructions. RNAs were isolated in order to measure the level of expression of MCEMP1 in the following cells: Daudi (a B lymphoblast cell line derived from Burkitt's lymphoma, ATCC No. CCL-213), THP-1 (a monocytic leukemia cell line,

ATCC No. TIB202), TF-1 (a myeloid progenitor cell line, ATCC No. CRL-2003), HMC-1, (a mast cell line); primary monocytes; primary B cells; primary basophils; CD34+ progenitor cells; *in vitro* cultured mast cells at week 5 and week 9; macrophages and macrophages activated by LPS; HPB-ALL, (a T cell leukemia cell line); primary lymphocytes; neutrophils; and; primary human vascular endothelial cells (HUVAC).

Equal amounts of each of the RNAs from the cell lines indicated above were used as PCR templates in reactions to obtain the threshold cycle (C_t). The C_t was normalized using the known C_t from 18S RNAs to obtain ΔC_t. To compare relative levels of gene expression of MCEMP1 in different cell lines, $\Delta\Delta C_t$ values were calculated by using the lowest expression level as the base, which were then converted to real fold expression difference values. MCEMP1 mRNA was found to be expressed in week 5 and week 9 in vitro cultured mast cells. Moderate levels were found in monocytes (Fig. 3). Among the five human tissues examined, MCEMP1 was highly expressed in the lung, but very little expression was observed in heart, liver, brain, trachea, and kidney (Fig. 3).

Example 2

Expression of MCEMP1 Protein

To determine the MCEMP1 gene product, MCEMP1 cDNA was PCR-amplified by using two oligo primers:

5'CACCATGGACTACAAAGACGATGACGACAAGGAAGTGGAGGAAATCTACAAGC, (SEQ ID NO:9), and

5'TTGAGGTGAGGACTGTGGCATTT (SEQ ID NO:10),

and cloned into pcDNA3.1D/V5-His vector (Invitrogen) with an N-terminal Flag tag sequence attached to the N-terminus of MCEMP1 and a V5 tag fused to the C-terminus. The resultant clone, pMCEMP1-FV, was transiently transfected into 293T cells. Forty hours after transfection, transfected cells were harvested and separated into membrane and cytosolic fractions by either a homogenization or freeze-thaw method. Western blot analysis was performed using anti-Flag or Anti-V5 MAb and anti-mouse IgG conjugates. MCEMP1 was expressed as a predominant 35 kDa protein. Minor forms of 29 and 32 kDa proteins were also present in MCEMP1 transfected cells. The fact that all the protein bands were larger than the calculated molecular weight, 21 kDa, implies that MCEMP1 might be post-translationally modified, e.g., by glycosylation. Computer-assisted sequence analysis by PROSITE motif scan showed that MCEMP1 has a putative N-glycosylation site at aa 40-43. Fractionation of cells resulted in the presence of MCEMP1 in the membrane fraction, but very little was present in the cytosol (Fig. 3).

Example 3

Cell Surface Expression of MCEMP1

To further confirm that MCEMP1 is expressed on the cell surface and determine which terminus is on the outside of the cellular membrane, MCEMP1-transfected cells were stained with either FITC-conjugated anti-Flag or anti-V5 mAb and analyzed by fluorescent microscopy and by flow cytometry. While MCEMP1 was detected on the membrane with flow cytometry analysis by anti-Flag mAb, anti-V5 mAb was not (Fig. 5). A similar result was obtained from fluorescent microscopic assessment that MCEMP1-transfected cells were stained bright, especially on the outer surface of living

cells, by FITC-conjugated anti-Flag mAb, but not by the anti-V5 mAb. These results indicate that MCEMP1 is a transmembrane protein with the N-terminus exposed on the outside of the cellular membrane.

Administering MCEMP1-binding Molecules

The antagonistic or agonistic MCEMP1 binding molecules, such as antibodies and biologically active fragments thereof, of the present invention can be administered to patients in an appropriate pharmacological formulation by a variety of routes, including, but not limited to, intravenous infusion, intravenous bolus injection, and intraperitoneal, intradermal, intramuscular, subcutaneous, intranasal, intratracheal, intraspinal, intracranial, and oral routes. Such administration enables them to bind to endogenous MCEMP1 and inhibit/stimulate the action MCEMP1. These antagonists can also block the binding of the natural ligand for MCEMP1.

The estimated dosage of such antibodies is between 10 and 500 µg/ml of serum. The actual dosage can be determined in clinical trials following the conventional methodology for determining optimal dosages, *i.e.*, extrapolating a dosage range from *in vitro* and *in vivo* experiments, and then administering various dosages within the range to determine which is most effective.

It should be understood that the foregoing description is exemplary only, and not limiting, and the invention is defined only in the claims which follow, and includes all equivalents, known and unknown, of the claimed subject matter.

What Is Claimed Is:

1. An isolated nucleic acid molecule having at least 80% sequence identity to:

   (a) a nucleotide sequence encoding amino acid residues 1 to 187 of SEQ ID NO:2,

   (b) a nucleotide sequence of SEQ ID NO:1, or

   (c) the full-length coding sequence of nucleotide sequence SEQ ID No 1.

2. A vector comprising the isolated nucleic acid molecule of claim 1.

3. A host cell comprising the vector of claim 2 operably linked to regulatory sequences suitable for expression of the nucleic acid in the host cell.

4. The host cell of claim 3, which is a mammalian cell, a bacterial cell, a yeast cell, or a Baculovirus-infected insect cell.

5. A process for producing a MCEMP1 polypeptide comprising culturing the host cell of claim 3 under conditions suitable for expression of MCEMP1 polypeptide and recovering MCEMP1 polypeptide from the cell culture.

6. An isolated nucleic acid molecule that hybridizes under stringent hybridization and washing conditions to:

   (a) a nucleotide sequence encoding amino acid residues 1 to 187 of SEQ ID NO:2,

   (b) the complement of (a),

   (c) a nucleotide sequence shown as SEQ ID NO:1, or

   (d) the full-length coding sequence for MCEMP1 of SEQ ID No.1.

7. A vector comprising the nucleic acid molecule of claim 6.

8. A host cell comprising the vector of claim 7.

9. The host cell of claim 8, which is a mammalian cell, a bacterial cell, a yeast cell, or a Baculovirus-infected insect cell.

10. A process for producing a MCEMP1 polypeptide comprising culturing the host cell of claim 8 under conditions suitable for expression of said polypeptide and recovering said polypeptide from the cell culture.

11. An isolated polypeptide having at least 80% sequence identity to SEQ ID NO:2 or a biologically active fragment or variant thereof, or a derivative thereof.

12. An agonist or antagonist antibody, which specifically binds to an MCEMP1 polypeptide or biologically active fragment or variant thereof.

13. The agonist or antagonist of claim 12, wherein the MCEMP1 polypeptide comprises amino acid residues 1 to 187 of SEQ ID NO:2.

14. An agonist or antagonist antibody, which binds to the polypeptide of claim 11.

15. The agonist or antagonist antibody of claim 12, wherein said antibody is a monoclonal antibody.

16. The agonist or antagonist antibody of claim 12, which is labeled.

17. The agonist or antagonist antibody of claim 12, which is immobilized on a solid support.

18. A composition comprising an agonist or antagonist antibody of claim 12 in admixture with a pharmaceutically acceptable carrier.

19. The agonist or antagonist antibody of claim 12, which is bispecific.

20. The agonist or antagonist antibody of claim 12, which is chimeric, humanized, or human.

21. The agonist or antagonist antibody of claim 12, which is an antigen binding fragment or a single-chain antibody.

22. The agonist or antagonist antibody of claim 20, wherein the antigen binding fragment is selected from the group consisting of: Fab, Fab', $F(ab')_2$, and Fv.

23. A method for inhibiting the action of MCEMP1 comprising administering an antagonist antibody of claim 12.

24. A method of stimulating the action of MCEMP1 comprising administering an agonist molecule of claim 12.

25. A method of treating an immunological disease associated with MCEMP1 comprising administering an antibody of claim 12.

26. A method to screen molecules or compounds for their ability to form complexes with MCEMP1, the method comprising:

(a) combining MCEMP1 with the compound or molecule under conditions to allow complex formation; and

(b) detecting complex formation, wherein the presence of the complex identifies a molecule or compound that specifically binds MCEMP1.

27. The method of claim 26, wherein the molecules or compounds are chosen from the group consisting of peptides, agonists, antagonists, inhibitors, antibodies, and pharmaceutical agents.

28. A method of inhibiting mast cell activation comprising administering an antibody of claim 12.

Figure 1. Nucleotide Sequence (SEQ ID No.1) and the deduced amino acid sequence (SEQ ID No.2) of a cDNA encoding the MC[redacted]P1

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GGGGTGGAGC TGAGGGGTGG AGCTGAGGCT GGAGGGAGGA AGGTGTGGGG GGACCCAGGG GTCCTGTCTC CAAGCCTGGT TGCTCTTACG CGAAAAGTTG GGACACTGAG      110

GTGTCACAGC TTCTCTTTTG AAATGGAGAG GAGGTAGGAG GGTGAGGTCC ATCCAGGTAG ACACAGACAC ACACAGAGAC CACAGCTTCC TGTAACATTT CCGAGTGTCG      220

AATTCCATCT CCCGGTCTAG AGGTTTTTCT TCTTGGTCCT TCCTGAGACC TCTTGGCTCC CAAGAGCCTC TTGATCGGGG CAGGAATGAG GGTGCCCCAG GGTGCGAGAG      330

TCGTGGATCC CTGAAAAGAG GAGGCTGCTC CCCCTCTTTC TTCCCCCCAC CTCCAGATTT CCTCATCTGC CCACACCTTC CGGTGGGCGG GGACGTGTAT GGACAAATTT      440

GCGGGCTGGGGACC ATG GAA GTG GAG GAA ATC TAC AAG CAC CAG GAA GTC AAG ATG CAA GCA CCA GCC TTC AGG GAC AAG AAA CAG GGG GTC TCA      535
               Met Glu Val Glu Glu Ile Tyr Lys His Gln Glu Val Lys Met Gln Ala Pro Ala Phe Arg Asp Lys Lys Gln Gly Val Ser

GCC AAG AAT CAA GGT GCC CAT GAC CCA GAC TAT GAG AAT ATC ACC TTG GCC TTC AAA AAT CAG GAC CAT GCA AAG GGT GGT CAT TCA CGA CCC      628
Ala Lys Asn Gln Gly Ala His Asp Pro Asp Tyr Glu Asn Ile Thr Leu Ala Phe Lys Asn gln Asp His Ala Lys Gly Gly His Ser Arg Pro

ACG AGC CAA GTC CCA GCC CAG TGC AGG CCG CCC TCA GAC TCC ACC CAG GTC CCC TGC TGG TTG TAC AGA GCC ATC CTG AGC CTG TAC ATC CTC      721
Thr Ser Gln Val Pro Ala Gln Cys Arg Pro Pro Ser Asp Ser Thr Gln Val Pro Cys Trp Leu Tyr Arg Ala Ile Leu Ser Leu Tyr Ile Leu

CTG GCC CTG GCC TTT GTC CTC TGC ATC ATC CTG TCA GCC TTC ATC ATG GTG AAG AAT GCT GAG ATG TCC AAG GAG CTG CTG GGC TTT AAA AGG      814
Leu Ala Leu Ala Phe Val Leu Cys Ile Ile Leu Ser Ala Phe Ile Met Val Lys Asn Ala Glu Met Ser Lys Glu Leu Leu Gly Phe Lys Arg

GAG CTT TGG AAT GTC TCA AAC TCC GTA CAA GCA TGC GAA GAG AGA CAG AAG AGA GGC TGG GAT TCC GTT CAG CAG AGC ATC ACC ATG GTC AGG      907
Glu Leu Trp Asn Val Ser Asn SER Val Gln Ala Cys Glu Glu Arg Gln Lys Arg Gly Trp Asp Ser Val Gln Gln Ser Ile Thr Met Val Arg

AGC AAG ATT GAT AGA TTA GAG ACG ACA TTA GCA GGC ATA AAA AAC GTT GAC ACA AAG GTA CAG AAA ATC TTG GAG GTG CTG CAG AAA ATG CCA     1000
Ser Lys Ile Asp Arg Leu Glu Thr Thr Leu Ala Gly Ile Lys Asn Val Asp Thr Lys Val Gln Lys Ile Leu Glu Val Leu Gln Lys Met Pro

CAG TCC TCA CCT CAA TAA ATGAGAGGAC ATTGTGGCAG CCAAAGCCAC AACTTGGAAG ATGGGGCTGC ACCTGCCAAC GAAGACGGGA AATGACCCCC CCCCCAGCCT     1108
Gln Ser Ser Pro Gln  *

AGTGTGAACC TGCCCCTCGT CCCACGTATA GAAAAACCTC GAGTCATGGT GAATGAGTGT CTCGGAGTTG CTCGTGTGTG TGTACACCTG CGTGCGTGTG TGTGCGTGTG     1218

TGCGCGTGTG TTCGTGTGTG TGCGTGTGTG CGTGCGCGTG TGTGTGCATT TTGCAAAGGG TGGACATTTC AGTGTATCTC CCAGAAAGGT GATGAATGAA TAGGACTGAG     1328

AGTCACAGTG AATGTGGCAT GCATGCCTGT GTCATGTGAC ATATGTGAGT CTCGGCATGT CACGGTGGGT GGCTGTGTCT GAGCACCTCC AGCAGATGTC ACTCTGAGTG     1438

TGGGTGTTGG TGACATGCAT TGCACGGGCC TGTCTCCCTG TTTGTGTAAA CATACTAGAG TATACTGCGG CGTGTTTTCT GTCTACCCAT GTCATGGTGG GGGAGATTTA     1548

TCTCCGTACA TGTGGGTGTC GCCATGTGTG CCCTGTCACT ATCTGTGGCT GGGTGAACGG CTGTGTCATT ATGAGTGTGC CGAGTTATGC CACCCTGTGT GCTCAGGGCA     1658

CATGCACACA GACATTTATC TCTGCACTCA CATTTTGTGA CTTATGAAGA TAAATAAAGT CAAGGGAAAA AAAAAAAAAA AAAAAAAAA                           1747
```

Figure 2. Genomic structure and alternative splicing of MCEMP1.

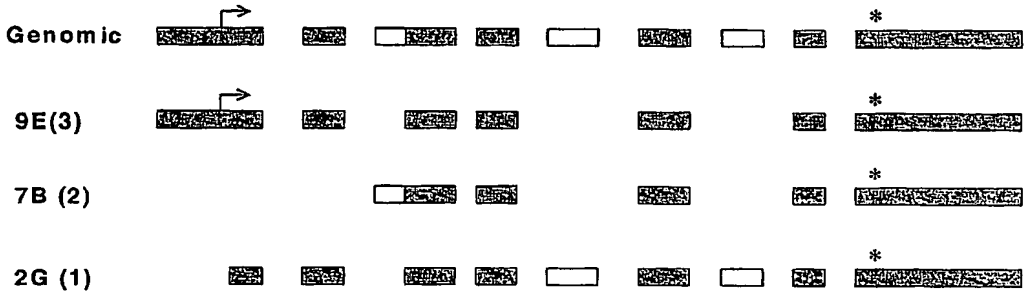

Figure 3A. Relative expression (real fold difference) of MCEMP1 in tissues

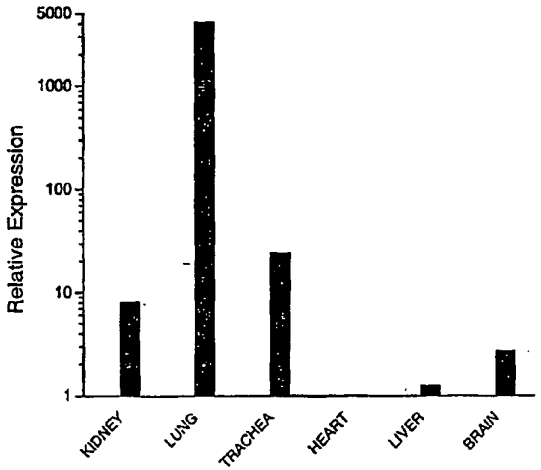

Figure 3B. Relative expression (real fold difference) of MCEMP1 in primary cells and cell lines.

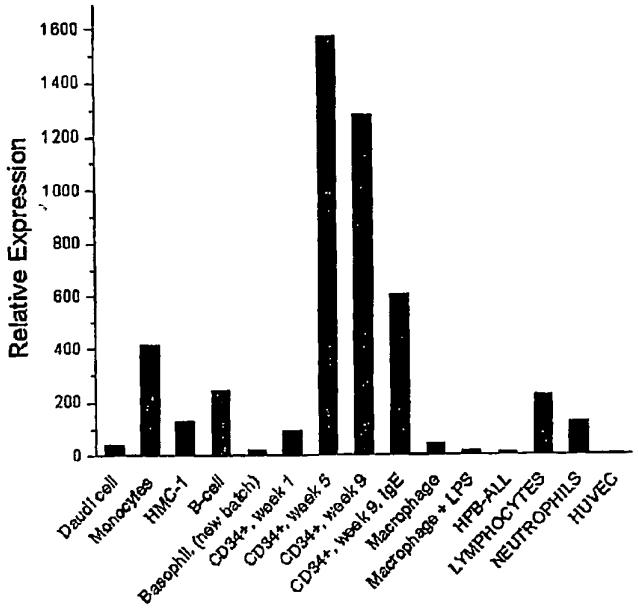

Figure 4. Expression of MCEMP1-FV in transfected 293T cells and cellular membrane fraction

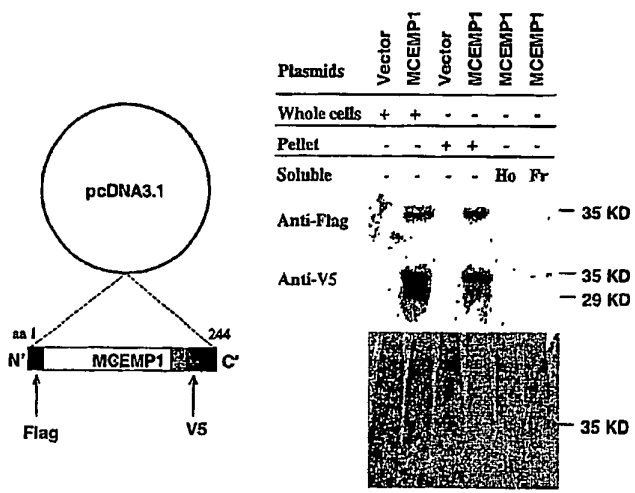

Figure 5. Cell surface expression of MCEMP1-FV. Empty vector or MCEMP1 transfected cells (1 x 10^6) were preincubated with at room temperature with 100 degree C of FACS buffer containing 2% normal rabbit serum to block nonspecific binding. Anti-Flag, or Anti-V5 was added at 10 micrograms/ml and incubated at room temperature for 30 min. After extensive washing, cells were stained with FITC-conjugated anti-mouse IgG (Becton Dickinson and Company, Mountain View, California) in FACS buffer. Cells were then washed and a minimum of 5,000 cells were analyzed by a FACScan (Becton Dickinson).

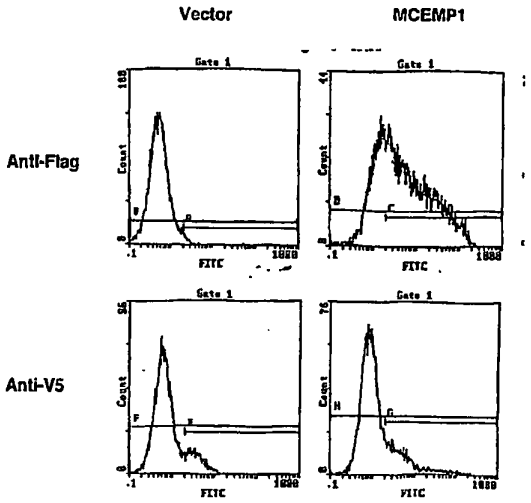

Table 1: Allelic Variations of MCEMP1

Sequence	ATT	GTT
Aminoacid (167)	Ile	Val
Frequency	Ile	Val
cDNA	5	2
ESTs	8	1

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